Immunity, Vol. 6, 97–105, January, 1997, Copyright ©1997 by Cell Press

Editing Disease-Associated Autoantibodies

Ching Chen,* Eline Luning Prak,* and Martin Weigert
Department of Molecular Biology
Princeton University
Princeton, New Jersey 08544

Summary

We have generated site-directed transgenic mice whose transgenes code for anti-DNA antibodies. These antibodies are representative of the lupus-associated anti-DNAs seen in mouse models of autoimmunity and human SLE, and have the usual characteristics of pathogenic autoantibodies. As conventional transgenics in nonautoimmune mice, anti-DNA B cells have been shown to be deleted or inactivated. Autoreactive B cells can also escape negative regulation by a process called receptor editing. Here we describe two combined immunoglobulin H and L chain site–directed transgenic mouse models and characterize their editing phenotypes. One model, 3H9R/Vκ4R, has a deletion-prone phenotype and undergoes editing, primarily by inactivation of the light chain by leapfrogging events. In the other model, 3H9R/Vκ8R, B cells are susceptible to anergy and maintain most of their HR and LR chains. These studies clarify the relationship between editing and other mechanisms of tolerance.

Introduction

Editing is a process by which the specificity of the B cell antigen receptor is modified by furthur V gene rearrangement. Such secondary rearrangements were proposed to explain features of light (L) chains in transgenic mice whose transgenes code for autoantibodies. Anti-H-2^k transgenics with an H-2^k allele have an increased frequency of λ L chain B cells (Tiegs et al., 1993); editing of anti-DNA transgenics leads to high frequencies of Jκ5 (Chen et al., 1994; Radic et al., 1993a). To study editing directly, we have placed rearranged VL and VH genes into their native contexts (Chen et al., 1995a; Luning Prak and Weigert, 1995). Unlike earlier transgenic models, these rearranged L and heavy (H) chain genes are equipped with upstream V genes. In the L chain site-directed transgenic (sd-tg or LR), the Jκ locus was replaced by the Vκ4Jκ4–Jκ5 gene (Vκ4R). We showed that the Vκ4R gene could be displaced by further rearrangements involving upstream Vκ genes and the downstream Jκ5 segment (Luning Prak and Weigert, 1995). In the case of the H chain sd-tg, 3H9R, the JH locus was replaced by a VDJ, which has a conserved heptamer/nonamer. This VH gene is replaced by upstream genes at the embedded recombination signal (Chen et al., 1995a).

That secondary rearrangements occur at 3H9R and Vκ4R is surprising, since functional H and H/L pairs are commonly thought to stop further rearrangement at the H and L chain loci, respectively. However, in an HR animal some HR endogenous L chain combinations are likely to be autoreactive; similarly, in LR-only animals, some LR/endogenous H chain combinations are likely to be autoreactive. Editing may be instigated in these B cells; however, this is difficult to establish without knowing whether the edited specificities were in fact autoreactive. To test the role of autoreactivity in initiating editing, we have intercrossed HR and LR mice to generate two different anti-DNA sd-tgs, 3H9R/Vκ4R and 3H9R/Vκ8R. Both H/L combinations have characteristics of spontaneous anti-DNAs seen in lupus mice (Radic and Weigert, 1994; Shlomchik et al., 1990). Both are regulated in normal mice as shown in "conventional" transgenics: 3H9/Vκ4 transgenic B cells are deleted or edited (or both), while 3H9/Vκ8 cells are inactivated (Erikson et al., 1991). If editing is driven by autoreactivity, then we would predict that the frequency of secondary rearrangement at HR or LR (or both) would be higher in this setting than in either HR or LR alone.

If this were the case, these models of autoimmunity would allow us to address two additional questions: is the relative efficiency of H versus L chain editing the same, and does the difference in the regulation of 3H9R/Vκ8R and 3H9R/Vκ4R extend to editing?

Results

Our experimental approach depends on hybridomas from 3H9R/Vκ4R and 3H9R/Vκ8R mice. The hybridomas were derived from lipopolysaccharide (LPS)-activated splenic B cells and in this regard are comparable with previous hybridoma surveys from conventional transgenics and the individual Vκ4R and Vκ8R sd-tg mice. Hybridomas were analyzed for H and L chain genotype and for single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) binding activity. These properties were compared with the earlier hybridoma libraries (summarized in Table 1).

Evidence that these sd-tgs are actively regulated can be seen in that virtually no anti-dsDNA hybrids were found. This feature is also reflected in the lack of serum anti-DNA activity (data not shown). Despite having normal levels of serum immunoglobulin G (IgG) and IgM, neither 3H9R/Vκ4R nor 3H9R/Vκ8R mice show more than background levels of DNA binding activity. The virtual absence of anti-DNA antibodies indicates that the specificity of the transgene-encoded antibodies was modified. We find, first, that most surviving 3H9R/Vκ4R B cells have been edited, while most 3H9R/Vκ8R B cells have not; second, that L chains rather than H chains serve as the major targets in successfully edited 3H9R/Vκ4R B cells; and third, that the major L chain editing pathway in 3H9R/Vκ4R B cells involves inactivation of the Vκ4R gene by rearrangement of upstream Vκ12/13 genes to the downstream Jκ5 segment. To arrive at these conclusions, we have analyzed the rearrangement status of the H and the κ chain loci in splenic hybridomas from 3H9R/Vκ4R and 3H9R/Vκ8R mice.

* These authors contributed equally to this study.

Table 1. Genotypes and DNA-Binding Properties of LPS Hybridomas from tg and sd-tg Lines of Mice

	sd-tg				tg	
	3H9R/Vκ4R	3H9R/Vκ8R	Vκ4R	Vκ8R	3H9/Vκ8	3H9/Vκ4
DNA binding						
ssDNA	55 (38%)	6 of 6[b]	NT[a]	NT	50 (84%)	NT
dsDNA	2 (2%)	0 of 6[b]	NT	NT	0	0[c]
tg Status						
3H9 VH$^+$	143 (98%)	66 (89%)	NT	NT	50 (84%)	62 of 72 (86%)[d]
Vκ$^+$	21 (15%)	73 (98%)	44 (76%)	36 (94%)	56 (93%)	19 of 19 (100%)[e]
Total	146	74	58	38	60	

Hybridoma data are given for six different strains of mice; four are site-directed transgenics, sd-tg, and the remaining two are conventional immunoglobulin transgenics, tg. Shown are the numbers of B cell hybridomas with the different phenotypic and genotypic characteristics. DNA binding activities of hybridoma supernatants were determined by solution-phase ELISA, as described in the Experimental Procedures. 3H9 VH$^+$ refers to the presence of the 3H9 H chain DNA, tested by PCR (see Experimental Procedures). Vκ$^+$ indicates that the Vκ4 or Vκ8 DNA was present and intact (see Experimental Procedures for a description of the L chain PCR). All hybridomas were derived from in vitro LPS-activated splenocytes. The origins of tested clones are as follows: 3H9R/Vκ4R, two mice (this paper); 3H9R/Vκ8R, two mice (this paper); Vκ4R only, two mice (Luning Prak and Weigert, 1995); Vκ8R only, one mouse (Luning Prak and Weigert, 1995); 3H9/Vκ8, one mouse (Erikson et al., 1991); and 3H9/Vκ4, two mice (Gay et al., 1993). All hybridomas from the 3H9R/Vκ4R and 3H9R/Vκ8R sd-tg mice secrete IgM/κ antibodies as determined by solid-phase ELISA. No λ-producers are identified.

[a] NT, not tested.

[b] Six 3H9R/Vκ8R clones were typed for ssDNA and dsDNA binding. As the results for all six were the same, no additional samples were tested.

[c] The 3H9/Vκ4 antibodies were examined for DNA binding by ANA staining assay, and none of the 228 monoclonal antibodies tested had a 3H9/Vκ4-like ANA pattern (Gay et al., 1993).

[d] The expression of 3H9 transgene by these hybrids was tested by an anti-idiotype reagent specific for the 3H9 H chain (Gay et al., 1993).

[e] In the Vκ4 transgene survey, 19 of 19 hybrids tested had detectable levels of Vκ4 mRNA (Gay et al., 1993).

Analysis of H Chain Gene Rearrangements in 3H9R/Vκ4R and 3H9R/Vκ8R B Cells

The specificity of 3H9R/Vκ8R and 3H9R/Vκ4R antibodies could be modified by receptor editing at either the H or the L chain locus. To characterize H chain editing in these models, we analyzed rearrangements on the targeted and the untargeted H chain alleles. H chain rearrangements occurring on the targeted allele would be expected to inactivate 3H9R by VH replacement, in which an upstream VH segment replaces the 3H9 VH by rearrangement to a recombination signal sequence embedded within the 3′ region of the V (Chen et al., 1995a). Alternatively or in addition, H editing rearrangements may involve the untargeted allele. Rearrangement on the untargeted allele may be necessary if inactivating rearrangements on the targeted H chain fail to produce an appropriate H chain. Another possibility is that the targeted H chain is left intact and the untargeted allele undergoes rearrangement. In the latter case, the H chain encoded by the untargeted allele must outcompete the 3H9R H chain for pairing with the L chain. This form of phenotypic editing, in which allelic inclusion occurs, has been described for L chain (Gay et al., 1993).

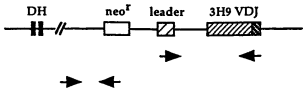
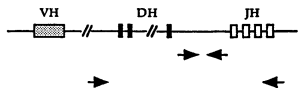
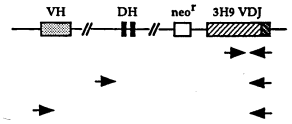
Allelic Inclusion

Is the 3H9 H chain edited by allelic inclusion? To address this possibility, we examined the rearrangement status of the untargeted allele in 3H9R$^+$ hybrids. Two polymerase chain reaction (PCR) assays were performed. One, using primers upstream of J_{H1} (JH1UP), amplifies a sequence that is deleted during D–J rearrangement. Thus, a JH1UP product identifies unrearranged (germline) alleles. The other, employing a degenerate D_H 5′ primer and a J_{H4} primer, amplifies most D–J rearrangements. An amplification product by either assay would establish an incompletely rearranged allele, i.e., allelic exclusion. Of the 3H9 VH$^+$ hybrids from 3H9R/Vκ4R mice, 22% have the untargeted allele in the germline configuration and 35% have an incomplete D–J rearrangement (Table 2). The 3H9R/Vκ8R hybrids show similar frequencies of unrearranged and incompletely rearranged alleles. At most then, 43% of B cells might have a complete VDJ rearrangement at the untargeted locus. The actual frequency of cells with complete H chain rearrangements on the untargeted allele is much lower because we underestimate the frequency of D–J rearrangement in our PCR assays. Our D_H 5′/J_{H4} primer combination does not detect D–J_{H1} (they are too far apart), and D–J_{H2} rearrangements are obscured by the D–J_{H2} rearrangement of the hybridoma fusion partner SP2/0. If the four JH genes are equally distributed (Gu et al., 1990), then about 70% of the cells should have incomplete D–J rearrangements. This high frequency of unrearranged and incompletely rearranged configurations of the untargeted locus demonstrates efficient allelic exclusion by the 3H9 VH gene. Hence, allelic inclusion does not significantly account for the self-tolerance observed in these mice.

3H9 Inactivation

The status of the inserted VH 3H9 construct was assessed using two PCR assays. One employs primers complementary to the 3H9 leader sequence and to the characteristic 3H9 CDR3 region. These amplify the complete 3H9 VH and no other VH genes (data not shown). The other verifies that the location of the construct is correct using gene targeting PCR primers previously described (Chen et al., 1995a). The majority (98%) of the 3H9R/Vκ4R B cells gave the correct amplification bands in both PCR tests (Table 2), indicating that the loss of dsDNA binding activity in these cells is not due to deletion of the 3H9 VH gene. Similarly, most of the 3H9R/Vκ8R B cells (89%) retained the 3H9 gene (Table 2).

Table 2. IgH Rearrangements in 3H9R/Vκ4R and 3H9R/Vκ8R B Cells

	3H9R/Vκ4R Hybrids		3H9R/Vκ8R Hybrids		Locations of Primers
	Tested	Positives	Tested	Positives	
Total Hybrids					
LD–CDR3	146	143 (98%)	74	66 (89%)	
Target	146	143 (98%)	74	66 (89%)	
3H9R$^+$ Hybrids					
JH1UP	143	31 (22%)	66	16 (24%)	
DH 5′–JH	143	50 (35%)	66	25 (38%)	
3H9R$^-$ Hybrids					
CDR2–CDR3	3	0	8	0	
DH 5′–CDR3	3	0	8	1 (12%)	
VHFW1–CDR3	3	2 (67%)	8	7 (88%)	

Hybridomas are the same as in Table 1. Schematic drawings of the targeted (top and bottom) and untargeted (middle) H chain alleles are shown on the right. Locations of each pair of PCR primers are indicated by arrows. Hybrids that tested positive in LD–CDR3 PCR are abbreviated as 3H9R$^+$ and hybrids that tested negative as 3H9R$^-$.

3H9 VH Replacement

Some VH replacement is still observed: 11% of 3H9R/Vκ8R and 2% of 3H9R/Vκ4R hybridomas. The difference (11% versus 2%) is significant ($\chi^2 = 7.92$, $p < 0.005$). The higher frequency in 3H9R/Vκ8R could be the result of less successful L chain editing due to the lack of downstream Jκ segments on the Vκ8R allele. A reduced efficiency of Vκ8R editing may amplify 3H9R/Vκ8R B cells, which are then edited at the H chain locus. Alternatively, the increased frequency of H chain inactivation in 3H9R/Vκ8R B cells may reflect a superior ability on the part of the Vκ8R L chain to pair with or produce (or both) nonautoreactive antibodies with edited H chains. Either explanation is consistent with earlier data obtained in Vκ4R and Vκ8R mice (Luning Prak and Weigert, 1995). In these L chain–only replacement mice, the frequency of Vκ4R editing was substantially greater than the frequency of Vκ8R editing, as evidenced by inactivating rearrangements of Vκ4R and skewing toward distal Jκ segment usage on the untargeted allele.

To characterize further the nature of the VH inactivation, we have localized the site of VH disruption in hybridomas that fail to amplify with leader CDR3 primers. All of these 3H9R$^-$ hybrids are also negative in a PCR assay using 3H9 CDR2 and CDR3 primers (Table 2). This means that the 3H9 VH is truncated at a site within the region encompassing these primers. To determine whether the disruption of 3H9R is due to invasion by upstream DH or replacement with VH genes, we examined the hybrids using two additional PCR assays (Chen et al., 1995a). These show that one hybrid had a DH invasion, but most of the cells with VH inactivations have undergone VH invasion (Table 2). This agrees with our previous finding that the disruption of 3H9 VH is mediated by a signal sequence embedded at the 3′ end of the 3H9 VH (and most other VH) genes (Chen et al., 1995a). Examination of the rearrangement status on the nontargeted allele revealed that at least three 3H9$^-$ hybridomas had incomplete D–J rearrangements (data not shown). This shows that the V to VDJ recombination on the targeted allele gave rise to the functional VH gene in these three antibody-secreting lines.

In sum, the analysis of H chain rearrangements reveals that only a minority of 3H9R/Vκ8R and 3H9R/Vκ4R B cells undergo H chain editing. There appears to be little or no role for H chain allelic inclusion, and inactivation by D invasion or VH replacement appears to occur infrequently in surviving B cells. Hence, most B cells have escaped tolerance by L chain editing or other forms of negative regulation. To analyze the contribution of L chain editing to the self-tolerant phenotype of these cells, we assayed rearrangements on the targeted and the untargeted κ alleles.

Analysis of κ L Chain Gene Rearrangements in 3H9R/Vκ4R and 3H9R/Vκ8R B Cells

The rearrangement status of the κ genes in 3H9R/Vκ4R and 3H9R/Vκ8R hybrids was examined in a series of PCR assays. Hybrids were first tested for the presence of Vκ4R or Vκ8R genes using 4R- or 8R-specific primers (Luning Prak and Weigert, 1995). Several pairs of primers were then used to test for additional rearrangements on the targeted and untargeted κ alleles. The Vs, a degenerate Vκ primer that binds 80%–90% of Vκ genes, and the Jκ5 primer combination detects most Vκ rearrangements to Jκ4 and Jκ5. The Vs and Jκ2 primers were used to detect Jκ1 rearrangements (Jκ1 rearrangements do not reliably amplify with the Vs and Jκ5 primers because of the large product size). Since the Vs primer amplifies a Jκ2 rearrangement in the fusion partner, SP2/0, a different, non-SP2/0-reactive Vκ primer (L5) was used to test for Jκ2 rearrangements (Luning Prak and Weigert, 1995). Jκ4 rearrangements can come from the inserted Vκ4R gene or from a new Vκ–Jκ4 rearrangement on the untargeted allele. Vκ4 and Jκ5 primers specifically amplify Vκ4R if it lies in proximity to Jκ5 and Cκ. For example, if a hybrid is positive in 4R PCR, is Jκ2, Jκ4, and Jκ5 positive in Vs–Jκ5 PCR, is Jκ4 and Jκ5 positive in L5–Jκ5 PCR, but is negative in Vκ4–Jκ5 PCR, then the genotype of this hybrid is 4R^{inv}/Jκ4, i.e.,

Table 3. Summary of L Chain Rearrangements in sd-tg B Cell Hybridomas

Genotype	3H9R/Vκ4R	Vκ4R	3H9R/Vκ8R	Vκ8R
R/0	5 (4)	11 (19)	53 (71)	16 (42)
R/1, 2, 4, 5	16 (11)	33 (57)	20 (27)	20 (52)
R^{inv}	84 (57)	7 (12)	NA	—
R^{del}	41 (28)	7 (12)	1 (2)	2 (6)
Total	146 (100)	58 (100)	74 (100)	38 (100)

The status of the targeted locus is indicated in the left column. R indicates that the Vκ4R or Vκ8R is present and in proximity to Cκ on the targeted locus (the Vκ4 plus Jκ5 PCR is positive). R^{inv} means that Vκ4R has been inverted by rearrangement to the downstream Jκ5 segment. Inversions are not applicable to Vκ8R or 3H9/Vκ8R clones because they lack an available downstream Jκ segment on the targeted κ locus. R^{del} clones lack Vκ4 (or Vκ8) DNA, but usually have amplifiable Jκ5 rearrangements. The numbers of LPS hybridomas with each L chain genotype are given in the column under each mouse line. Data from the L chain–only mice (Vκ4R and Vκ8R) have been published elsewhere (Luning Prak and Weigert, 1995). Percentage distributions within each strain (summed vertically) are given in parentheses.

Table 4. L Chain Rearrangements in 3H9R/Vκ4R Hybridomas

	Untargeted Locus Jκ Usage					
Targeted Allele	0	1	2	4	5	Total
4R	5	3	3	2	8	21
$4R^{inv}$	67	5	5	7	—	84
$4R^{del}$	26	8	7	0	—	41
Total	98	16	15	9	8	146
Targeted plus untargeted Jκ	98	16	15	30	133	292

Shown are the numbers of hybridomas with an unrearranged targeted locus (4R) or a targeted locus that has undergone a leapfrogging rearrangement to Jκ5 ($4R^{inv}$ or $4R^{del}$); these values are given in the total column on the right. In addition, the hybridomas are subclassified by Jκ rearrangement status on the untargeted allele. Zero indicates that no rearrangement was found on the untargeted locus; it is therefore presumed to be in the germline configuration. We note that the numbers of R/0, $4R^{inv}$/0, and $4R^{del}$/0 represent maximal estimates because our PCR assays do not always distinguish 4R/0 from 4R/4 or $4R^{inv}$/0 from $4R^{inv}$/5 or $4R^{inv}$/RS and $4R^{del}$/0 from $4R^{del}$/5 or $4R^{del}$/RS. Furthermore, the fusion partner, SP2/0, has a Jκ2 rearrangement that amplifies with the Vs primer. We have used a Vκ-specific primer (Vκ12/13; Luning Prak and Weigert, 1995) and the L5 Vκ primer (which does not amplify the SP2/0 Jκ2 rearrangement) to distinguish some of these genotypes. The bottom row gives the estimated total numbers of rearrangements to Jκ1, Jκ2, Jκ4, and Jκ5 for both the targeted and the untargeted alleles. The bottom row factors in the Jκ5 rearrangements on the targeted allele, as seen in the $4R^{inv}$ and $4R^{del}$ hybridomas (see Results). The hybridomas originate from two separate in vitro LPS fusions (see Experimental Procedures) and have been summed.

it has an inversional rearrangement to the downstream Jκ5 on the targeted allele and a rearrangement to Jκ4 on the untargeted allele, and the rearrangement to Jκ2 is attributed to SP2/0. Using similar reasoning, we were able to determine the genotypes of most of the hybrids. Hybrids lacking identifiable Jκ rearrangements on the untargeted allele are categorized as R/0. Our genotyping strategy does not distinguish R/0 clones from those that may have rearranged to heptamer sites (RS) in the Jκ–Cκ intron or rearrangements to indistinguishable Jκ segments (e.g., 4R/4 versus 4R/0) on the untargeted allele.

Hybridomas from 3H9R/Vκ4R and 3H9R/Vκ8R mice have two major categories of L chain configurations (Table 3). The first category lacks further rearrangements on the targeted allele and thus expresses the Vκ4R or Vκ8R L chain. Additional rearrangements in this category, if present, occur on the untargeted allele. In the second category, the Vκ4R or Vκ8R genes have been deleted or inverted by secondary rearrangement (R^{inv} and R^{del}). In 3H9R/Vκ4R mice, 15% of the cells belong to the first category (being R/0 or R/1, 2, 4, 5), and the rest have inactivated the Vκ4R gene by further rearrangements (Table 3). In 3H9R/Vκ8R mice, on the other hand, 98% of the cells have maintained the functional Vκ8R gene. Editing on the targeted allele, if successful, inactivates the autoreactive Vκ4 L chain and replaces it with a nonautoreactive L chain in one step. The alternative, rearrangement on the untargeted locus and editing the Vκ4 by L chain competition, appears to be little used. Instead, it appears that the leapfrogging form of editing is considerably more efficient than allelic inclusion. The rarity of 4R/1, 2, 4, and 5 cells in 3H9R/Vκ4R contrasts with earlier data on 3H9/Vκ4 transgenic animals (Gay et al., 1993) and may pertain to differences between the Vκ4 transgene and Vκ4 sd-tg. Table 3 also shows that the majority of leapfrogging rearrangements occur by inversion. This suggested that the Vκ segment(s) that could efficiently veto anti-DNA activity was in the inversional orientation.

Jκ Usage

The Jκ usage of L chain rearrangements in 3H9R/Vκ4R and 3H9R/Vκ8R hybridomas is summarized in Tables 4–6. Table 4 illustrates the Jκ usage in 146 hybridomas from 3H9R/Vκ4R mice. We analyzed 292 alleles for rearrangement: there were 21 intact Vκ4R rearrangements and 98 putative germline alleles. Of the 178 alleles with additional rearrangements (i.e., 292 − 98 − 21 + 5), 133 (75%) were to Jκ5 (Table 4). The distal Jκ usage is consistent with editing by Vκ4R inactivation in these cells, since Jκ5 is the only Jκ segment available for editing on the targeted allele. Of the 133 Jκ5 rearrangements, 125 occurred in $4R^{inv}$ or $4R^{del}$ clones. Thus, a maximum of 125 Jκ5 rearrangements took place on the targeted allele. However, some of the $4R^{inv}$ and $4R^{del}$ clones may have rearrangements to Jκ5 on the untargeted allele as well or, in the case of $4R^{del}$, they may have undergone RS deletion on the targeted locus and Jκ5 rearrangement on the untargeted locus. Nevertheless, the simplest interpretation of the data is that the majority of $4R^{inv}$ and $4R^{del}$ clones harbor a Jκ5 rearrangement on the targeted allele and no additional rearrangements on the untargeted allele.

In clones that have retained the replaced L chain, a bias toward Jκ5 rearrangements is not seen. The absence of distal Jκ bias suggests that these clones have not undergone multiple rearrangement attempts. If rearrangements on the untargeted locus are direct (rather than secondary), then we should not find traces of prior rearrangements in clones with distal Jκ rearrangements. To test this prediction, we typed five 4R/5 hybridomas for Jκ1 rearrangements using Vs and Jκ2 primers. This assay should detect Jκ1 rearrangements in cells that

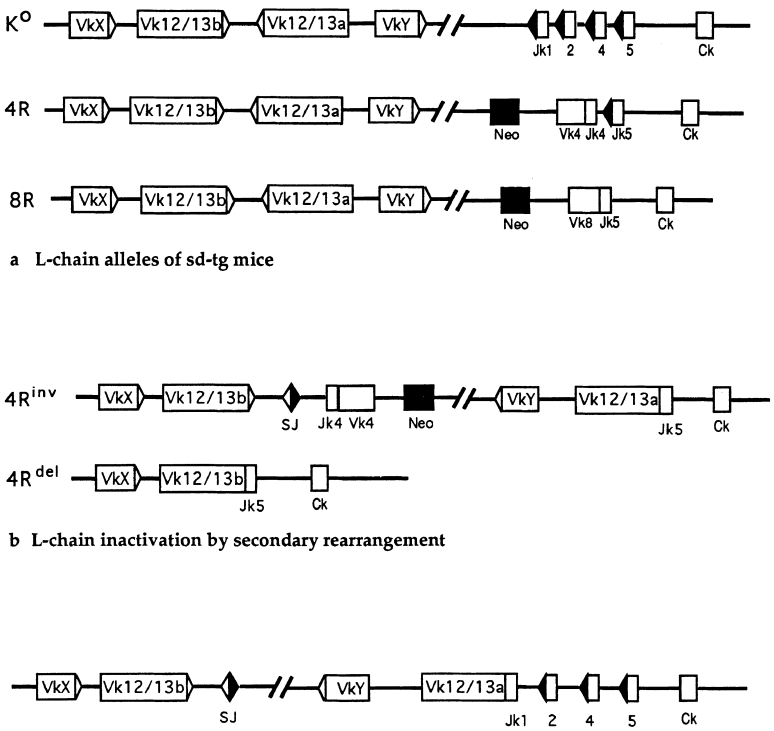


Figure 1. L Chain Editing Pathways in Anti-DNA B Cells

(a) The genotypes of the L chain sd-tg mice. 4R is the allele in which the Vκ4Jκ4–Jκ5 has replaced the germline Jκ locus, while 8R represents the Vκ8Jκ5 replacement. Each R allele is associated with a germline κ locus (κ0). These diagrams show an abbreviated Vκ locus that includes the orientations of the master editors, Vκ12/13 (a and b).

(b) The two major outcomes of editing in 3H9R/Vκ4R and how they depend on the orientation of the Vκ editor.

(c) A representative rearrangement on the untargeted allele (κ0). These are usually unedited and are referred to as direct rearrangements.

have undergone primary rearrangement to Jκ1, followed by secondary rearrangement by inversion to Jκ5. We find no Jκ1 rearrangements by Vs and Jκ2 PCR in any of five 4R/5 hybridomas tested (data not shown), consistent with the prediction that these Jκ5 rearrangements are direct events.

Vκ12/13 Usage among Edited L Chains in 3H9R/Vκ4R

Our earlier work in 3H9 transgenics (Gay et al., 1993; Radic et al., 1993a) demonstrated frequent Vκ12/13 rearrangements. The Vκ12/13 family is made up of approximately two to eight members based on Southern blot analysis (Kofler et al., 1989). Our own Vκ12/13 PCR assay recognizes at least three different Vκ12/13 family members (Luning Prak et al., 1994). Vκ12/13 L chains in association with the 3H9 H chain efficiently veto DNA binding (Ibrahim et al., 1995) and appear to be the most effective L chains for editing anti-DNA B cells (Luning Prak et al., 1994; Radic et al., 1993a). Consistent with our earlier findings, we note here that 68% of 3H9R/Vκ4R hybridomas analyzed had Vκ12/13 rearrangements and none bound dsDNA (data not shown). The Vκ12/13 gene(s) therefore appears to be the most successful L chain for editing. By characterizing the Jκ usage among Vκ12/13 rearrangements, the successful editing rearrangement pathways can be ascertained (see Table 5; Figure 1).

Table 5 shows, first, that the majority of Vκ12/13 rearrangements appear to occur by inversion. This can be appreciated by looking at the most common genotype category, $4R^{inv}$/0, in which 11 out of 14 clones tested by Vκ12/13 PCR have a Vκ12/13–Jκ5 rearrangement. In contrast, most of the $4R^{del}$/0 clones are negative for Vκ12/13. Therefore it appears that a particular Vκ12/13 segment(s), in the inversional orientation, is greatly preferred. This is illustrated in Figure 1. A second observation pertaining to the Jκ usage is that all five $4R^{inv}$/1 hybridomas have Vκ12/13 rearrangements to Jκ5 (Table 5). Similarly, among $4R^{inv}$/4 clones, all of the Vκ12/13 rearrangements are to Jκ5 (Table 5). As the Vκ12/13 rearrangement is the most efficient in rescuing B cells, it is probably productive and therefore likely occurred last. By this line of reasoning, we conclude that rearrangements on the targeted locus are slower than on the untargeted locus in these $4R^{inv}$/1 or $4R^{inv}$/4 cells. However, slow rearrangement is not intrinsic to the targeted locus, because the opposite result occurs in $4R^{del}$ hybridomas (Table 5). Here the Vκ12/13 rearrangements are occurring preferentially on the untargeted rather than the targeted allele. For example, all seven Vκ12/13-positive $4R^{del}$/1 clones have Vκ12/13 rearrangements to Jκ1 rather than to Jκ5. The simplest explanation for these findings is that inversion to a particular Vκ12/13 segment is the best way of rescuing 3H9 B cells and that inversion occurs more slowly than deletion.

L Chain Editing in 3H9R/Vκ8R B Cells

Table 6 shows that 53 out of 74 3H9R/Vκ8R B cell hybridomas have no additional L chain rearrangements (they have the 8R/0 genotype). Eight clones from 3H9R/Vκ8R have inactivated the 3H9 H chain (Table 2). Of the remaining 21 cells that do have additional L chain rearrangements, only 1 has deleted the Vκ8R gene, presumably by RS deletion (Table 6). The remaining 20 clones potentially express two different L chains, retaining the Vκ8R gene on the targeted allele and exhibiting L chain rearrangements on the untargeted allele (genotypes 8R/1, 2, 4, or 5; Table 6).

The frequency of additional L chain rearrangements

Table 5. Vκ12/13 Jκ Distributions in 3H9/Vκ4 Hybridomas by L Chain Genotype

	Vκ12/13 Negative	Vκ12/13 Positive				
Genotype	0	1	2	4	5	Total
4R/1	1	2	—	—	—	3
4R/2	—	—	3	—	—	3
4R/4	—	—	—	2	—	2
4R/5	3	—	—	—	4	7
Subtotal	4	2	3	2	4	15
$4R^{inv}/0$	3	—	—	—	11	14
$4R^{inv}/1$	—	—	—	—	5	5
$4R^{inv}/2$	—	—	4	—	—	4
$4R^{inv}/4$	—	—	—	—	6	6
subtotal	3	—	4	—	22	29
$4R^{del}/0$	15	—	—	—	9	24
$4R^{del}/1$	1	7	—	—	—	8
$4R^{del}/2$	—	—	6	—	1	7
Subtotal	16	7	6	—	10	39

We analyzed 88 hybridomas from 3H9R/Vκ4R mice for Vκ12/13 rearrangements using Vκ12/13 and Jκ5 primers (see Experimental Procedures). Hybridomas with different L chain genotypes were chosen for this analysis. These L chain genotypes, described in the text, are indicated in the first column. The targeted allele genotype is indicated to the left of the slash, and the untargeted allele Jκ usage is given to the right of the slash. The numbers of clones that lack identifiable Vκ12/13 rearrangements are given in the second column (column 0). The Vκ12/13-positive clones are subclassified according to Jκ usage (the Jκ segment in the Vκ12/13 rearrangement) in columns headed 1, 2, 4, and 5. This subdivision provides insight into which rearrangements are occuring on the targeted versus the untargeted allele (see text).

is considerably lower in 3H9R/Vκ8R than it is in Vκ8R-only B cells (see Table 3). The lower frequency of clones with additional L chain rearrangements may be due to a shorter window of opportunity for secondary rearrangement in HR/LR B cells than in LR-only B cells. Alternatively, it may mean that anergic B cells do not edit. We chose the 3H9R/Vκ8R system to address this issue based on the phenotype of the transgenics.

Table 6. Summary of L Chain Rearrangements in Vκ8R/3H9R Hybridomas

Panel	8R/0	8R/1	8R/2	8R/4	8R/5	Other	Total
65 series	27	5	0	0	2	1	35
70 series	26	6	2	2	3	0	39
Total	53 (71)	11 (15)	2 (3)	2 (3)	5 (7)	1 (1)	74 (100)
Vκ12/13	0 of 40	2 of 11	2 of 2	2 of 2	5 of 5	NT	11 of 60

The Jκ rearrangement status of 74 hybridomas from two different 3H9R/Vκ8R mice (65 series and 70 series) was assessed by several different PCR assays including Vκ8, Vs and Jκ5, Vs and Jκ2, L5 and Jκ5, and Vκ12/13 and Jκ5. These assays are described in detail elsewhere (Luning Prak et al., 1994; Luning Prak and Weigert, 1995). The first row of the table denotes the L chain genotypes; 73 of 74 clones have intact Vκ8R DNA, and over 70% of hybrids show no evidence of additional L chain rearrangements (8R/0). The number of clones in each genotype category is listed. Percentages are given in parentheses and summed horizontally. Vκ12/13 rearrangements are expressed as the number of positive clones divided by the total number of clones that was tested. Faint bands were counted as positive, although some of these may represent distantly related Vκ12/13 genes. NT, not tested.

Transgenic 3H9/Vκ8 B cells are able to proliferate to T cell–dependent and independent stimuli, but did not secrete antibody (Erikson et al., 1991). A similar phenotype is suspected in 3H9R/Vκ8R B cells based on the absence of serum ssDNA binding activity, even though nearly all hybridomas express ssDNA-binding antibodies. Paradoxically, serum IgM and IgG levels are not similarly reduced, indicating that a small proportion of B cells may have been edited.

To distinguish a lack of editing from a limited window of opportunity for secondary rearrangement, we analyzed the Vκ12/13 usage among additional rearrangements in 3H9R/Vκ8R hybridomas. Editing predicts Vκ12/13 usage. Alternatively, if rearrangements on the untargeted allele are occurring at an early stage of B cell development and prior to selection on the basis of immunoglobulin specificity, Vκ12/13 usage should not be prominent among the secondary rearrangements. In the latter case, one would predict that most of the additional rearrangements should be nonproductive. Furthermore, additional rearrangements that are productive should exhibit a greater Vκ diversity than the edited rearrangements that were seen in 3H9R/Vκ4R B cells.

Of the 20 3H9R/Vκ8R clones with demonstrable additional L chain rearrangements (8R/1, 2, 4, 5), 11 have Vκ12/13 rearrangements (Table 6). This result differs significantly from Vκ12/13 usage in the LR-only mice: no clone out of 20 surveyed from the Vκ4R- and Vκ8R-only hybridoma panels had a Vκ12/13 rearrangement (data not shown). Thus it appears that Vκ12/13 rearrangement is intimately connected with the negative regulatory influence of the 3H9 H chain. One explanation for the lower number of Vκ12/13 rearrangements in 3H9R/Vκ8R is that editing is not as critical as it is in 3H9R/Vκ4R. Another possibility is that 3H9R/Vκ8R B cells that have productively rearranged L chains other than Vκ12/13 are negatively selected in the context of 3H9 H chain. Our findings in 3H9R/Vκ8R clarify our understanding of 3H9/Vκ4, in which Vκ12/13 appear to play a central role in editing and B cell survival. In 3H9/Vκ4, not only do rearrangements to Vκ12/13 predominate, but they typically inactivate the Vκ4 L chain.

Caveats

Most of the B cells that were analyzed did not produce anti-DNA antibodies. Escape from tolerance appears to be achieved mainly by L chain editing using Vκ12/13 (see above). However, not all B cells had identifiable rearrangements to account for their altered antibody specificity. A few clones have retained the 4R L chain. They seem to have escaped tolerance, or perhaps they have been edited in subtle ways that were not detected in our assays. Because the Vκ4R gene is at least grossly intact, the presence of 4R/1, 2, 4, or 5 cells (see Table 3 for example) may mean that L chain allelic inclusion can rescue a small proportion of 3H9R/Vκ4R B cells from deletion after all. Consistent with this interpretation, several of the 4R/1, 2, 4, or 5 clones have Vκ12/13 rearrangements and none of them binds dsDNA (data not shown). Another possibility is that these clones have undergone H chain editing. However, unexpectedly, most Vκ4R-positive clones appear to have intact 3H9R

DNA, and about half of them are positive for a D–J rearrangement or the germline configuration on the untargeted H chain allele (data not shown; see Table 2 for experimental design). Another possibility is that the 3H9R or Vκ4R genes may have been mutated in these cells. Undetected editing rearrangements or mutation may also account for the handful of "4R/0" clones with grossly intact 3H9 H chains, only one of which weakly binds dsDNA.

Discussion

We have created two monospecific sd-tg mice whose transgenes code for typical lupus-associated anti-DNAs. 3H9/Vκ4 arose spontaneously in a diseased MRL/lpr mouse and binds both ssDNA and dsDNA (Shlomchik et al., 1990). 3H9/Vκ8 is a combination that has also been seen in lupus mice; it only binds ssDNA (Radic et al., 1991; Radic and Weigert, 1994). Both autoantibodies have been studied as "conventional" transgenes. As such, 3H9/Vκ4 appears to be deleted in normal mice (Gay et al., 1993) and 3H9/Vκ8 has the characteristics of anergy (Erikson et al., 1991). As sd-tgs, both anti-DNAs are also negatively regulated. 3H9R/Vκ4R cannot be retrieved in fusions of LPS-activated B cells from HR/LR mice, suggesting that the 3H9R/Vκ4R B cell or its receptor is deleted. 3H9R/Vκ8R, however, is found at high frequencies in LPS fusions, but sera from these sd-tg mice have only background anti-DNA titers. A comparison of the conventional anti-DNA transgenes with the anti-DNA sd-tgs is presented in Table 1.

A key advantage of the sd-tg over the conventional transgene is the potential to study the role of editing in the establishment of self-tolerance. This was demonstrated in studies on the individual L chain and H chain genes that showed that each sd-tg can undergo secondary rearrangement (Chen et al., 1995a; Luning Prak and Weigert, 1995). The reason for secondary rearrangement of the individual sd-tgs is unclear, but it seems unlikely that these ongoing rearrangements result simply from the inability of the sd-tg to shut down rearrangement. Instead we favor the view that secondary rearrangements are activated or sustained by the self-specificity of the sd-tg product with certain endogenous partners. If activation of secondary rearrangement is due to signals generated by autorecognition, then we would expect an increased frequency of H or L chain replacement (or both) in these anti-DNA mice. This is clearly the case for 3H9R/Vκ4R; nearly every hybridoma from these mice shows evidence of L chain editing (Table 1). This is not the case for 3H9R/Vκ8R mice, in which most hybridomas retain and express both sd-tgs.

The 3H9R/Vκ4R mouse edits almost exclusively at L chain. This is not surprising for two reasons. First, the nature of VH replacement indicates that it often takes place early in B cell development. Many replacements show evidence for N addition (Chen et al., 1995a), a function thought to occur at the stage of H chain rearrangement and hence prior to when a tolerogenic signal would be received. Therefore, VH replacement may not play a major editing role, but instead may serve to diversify the VH repertoire. However, VH replacements have also been found that lack N additions (D. Ni, C. C., and M. W., unpublished data); thus an editing function at later stages of B cell maturation or in response to negative signals in the periphery is still possible. Second, editing just at the L chain fits the tenants of allelic exclusion as they apply to the termination of recombination. Although allelic exclusion is initiated in several ways, the maintenance of exclusion is apparently due to down-regulation of recombinase-activating genes (RAGs). Loss of RAG activity happens at two points, after an H chain is formed and again after a productive L chain rearrangement (Grawunder et al., 1995; R. R. Hardy, personal communication). These rearrangement shutdown points have been referred to as H-STOP and H/L-STOP (Cohn and Langman, 1990). Stop points were originally invoked to explain the high frequencies of unrearranged or incompletely rearranged H chain loci and germline κ and λ loci in B cells. That two stop points were thought to be necessary followed from the quasi-sequential order of rearrangement, first H and then L. Without H-STOP, a second H chain could potentially arise during the phase of L chain rearrangement. The waves of RAG expression and their correlation with checkpoints in B cell development are consistent with H-STOP and H/L-STOP. However, editing of autoreactive receptors requires that recombination be reinitiated or sustained. High RAG levels are in fact observed in mice enriched for autoreactive B cells, but as yet the timing of RAG expression during editing is not understood. Since the need to edit can only be perceived after H and L expression, the initial wave of RAG expression that finally leads to H-STOP is unlikely to be affected. Reinitiation of RAG after H/L-STOP would presumably reinitiate both H and L chain recombination, but editing of 3H9/Vκ4 is mainly of the L chain. Therefore, the most likely model is that editing suspends the H/L-STOP signal.

Given that editing sustains recombination, does the high frequency of unrearranged κ alleles (or for that matter germline λ loci) in κ-expressing B cells mean that editing plays a minor role in establishing B cell tolerance? This is a paradox reminiscent of that posed by the high κ:λ ratio of murine B cells. Instead of editing autoreactivity, λ recombination is thought to serve as a backup for aberrantly rearranged κ loci. Since aberrant rearrangement is frequent (Coleclough et al., 1981), much lower κ:λ ratios would be predicted. One solution employs "crash factors" (Coleclough, 1992), or a limit on the time allowed for a B cell to rearrange successfully. By limiting the window of rearrangement, a B cell may rarely have the opportunity to utilize the λ alternative before cell death.

In a similar sense, autoreactive B cells may have a limited window during which editing can occur, and if secondary rearrangement is unsuccessful, then autoreactive B cells will undergo deletion or inactivation. It has been estimated that the observed frequencies of $\kappa^+/\kappa 0$ B cells are compatible with two to three editing attempts (Arakawa et al., 1996; Nemazee, 1995). Alternatively or in addition, a narrow rearrangement window may be a consequence of the presence of a preformed H/L pair in sd-tg mice. Efficient delivery of the H/L-STOP signal, mediated by the H/L pair, may cause accelerated B cell

development and hence decreased opportunities for further rearrangement. The predominance of the leapfrogging pathway of L chain editing is attractive in the setting of a narrow window of opportunity because it promptly dispenses with the autoreactivity associated with the Vκ4R L chain. According to this narrow window model, nearly all cells with failed edited rearrangements on the targeted locus will die because they do not have the opportunity to rearrange a second time.

Several lines of evidence point to a limited window of opportunity for editing rearrangements in 3H9R/Vκ4R and 3H9R/Vκ8R B cells. First, as mentioned above, very few HR/LR B cells have more than one additional L chain rearrangement. For example, only 25% of $4R^{inv}$ and $4R^{del}$ clones have additional rearrangements on the untargeted locus (Table 4). Second, the Jκ usage among 4R/1, 2, 4, 5 and 8R/1, 2, 4, 5 clones is not biased toward the distal Jκ segments. This suggests that many of the editing rearrangements are primary. Analysis of 4R/5 clones is consistent with direct rearrangements; we do not detect the reciprocal products predicted by editing. Third, 4R/1, 2, 4, 5 and 8R/1, 2, 4, 5 clones have Vκ12/13 rearrangements, yet these Vκ12/13 rearrangements involve any Jκ segment, not just Jκ5. A marked Jκ5 bias is seen in 3H9 κ^{del}/wild-type B cells, in which most of the Vκ12/13 rearrangements on the untargeted allele occurred to Jκ5 (Luning Prak et al., 1994; E. L. P. and M. W., unpublished data). The decreased levels of leapfrogging on the untargeted κ allele in the double replacement system as compared with 3H9 κ^{del}/wild type can again be explained by shorter rearrangement windows. In 3H9R/Vκ4R B cells, the signal to delete may be given early in B cell development and the opportunity to edit may be limited by impending deletion. In contrast, 3H9 κ^{del}/wild-type cells have not yet rearranged their L chains and may therefore have more time to try out a few different L chains. Fourth, that no λ-expressing hybridomas were recovered in either HR/LR model (data not shown) suggests a limited window of opportunity for editing rearrangements. This interpretation must, however, be tempered with the knowledge that most λ l-chains are counterselected in the context of the 3H9 H-chain. Therefore the alternative, that the very low frequency of λ l-chains is due to negative selection, cannot be ruled out.

The HR/LR models reveal different roles for editing in deletional (3H9R/Vκ4R) and anergic (3H9R/Vκ8R) B cells. It is known that anti-dsDNA B cells undergo deletion in the bone marrow (Chen et al., 1995b). Here we show that editing, primarily in the form of further L chain recombination, often to Vκ12/13, can rescue anti-dsDNA B cells from death. As such, editing can play a role in central tolerance. The timing and role of editing in anergic cells is less clear; anergic cells may lack the ability to undergo editing at any stage, since 3H9R/Vκ8R B cells have fewer additional L chain rearrangements than 3H9R/Vκ4R B cells. Perhaps 3H9R/Vκ8R B cells pass through the filter of central tolerance without seeing the relevant self-antigen (or enough self-antigen) to initiate deletion and sustain recombination. Nevertheless, a small but significant fraction of 3H9R/Vκ8R are edited at either L or H. The L chain editing of this receptor is similar to that of the 3H9R/Vκ4R receptor, suggesting that some B cells with ssDNA specificity can accumulate enough antigen(s) to sustain recombination. Specifically, the preponderance of Vκ12/13 rearrangements suggests that editing can and does occur in the setting of anergy. H chain replacement, seen in 10% of 3H9R/Vκ8R hybrids, may represent reactivation of recombination in the periphery. The 3H9R/Vκ8R B cells may escape central tolerance but subsequently see a self-antigen for which the receptor has higher affinity or may reach a site at which the concentration of self-antigen is higher than in bone marrow. Thus, peripheral tolerance may reinitiate recombination and lead to more generalized editing.

Experimental Procedures

Mice

Generation of LR and HR sd-tg mice has been described previously (Chen et al., 1995a; Luning Prak and Weigert, 1995). Crosses between 3H9R and Vκ4R or Vκ8R mice gave rise to HR/LR double replacement mice. The presence of individual sd-tgs was identified by tail DNA PCR assays using established protocols (Chen et al., 1995a; Luning Prak and Weigert, 1995).

Generation of Hybridomas

Spleen cells from 2- to 4-month-old sd-tg mice were stimulated in vitro for 3 days with 20 μg/ml LPS (Sigma) and fused to SP2/0 myeloma cells using established procedures. The fusion was plated onto 96-well plates at limiting dilution. Only those hybrids from plates with less than 30 hybrids and with one microscopically visible colony per well were subjected to further analysis.

DNA Binding Assays

Binding to ssDNA and dsDNA was measured by a two-step solution phase enzyme-linked immunosorbent assay (ELISA) as described previously (Radic et al., 1993b). In brief, appropriate concentrations of antibody and biotinylated DNA were mixed, incubated at 37°C for 60 min, and transferred to microtiter plates coated with goat anti–mouse κ (Southern Biotechnology Associates). Following incubation at room temperature for 60 min, the plates were washed and bound antibody was detected with alkaline phosphatase–conjugated streptavidin. Antibody isotype and concentration were determined by solid-phase ELISA using an isotype-matched control antibody as a standard.

PCR Assays

Genomic DNA was prepared from individual hybrids as described (Luning Prak et al., 1994). Primers and conditions for H and κ chain PCR assays have been detailed previously (Chen et al., 1995a; Luning Prak et al., 1994; Luning Prak and Weigert, 1995).

Acknowledgments

The authors thank Shengli Wu, Donghui Ni, and Kim Ruch-Alegant for their excellent technical assistance in creating and maintaining the LR and HR mice; Donghui Ni for help with the figures; and Hui Xu, Michele Shannon, and Mark Shlomchik for thoughtful review of the manuscript.

Received September 19, 1996; revised December 10, 1996.

References

Arakawa, H., Shimizu, T., and Takeda, S. (1996). Re-evaluation of the probabilities for productive rearrangements on the κ and λ loci. Int. Immunol. *8*, 91–99.

Chen, C., Radic, M.Z., Erikson, J., Camper, S.A., Litwin, S., Hardy, R.R., and Weigert, M. (1994). Deletion and editing of B cells that express antibodies to DNA. J. Immunol. *152*, 1970–1982.

Chen, C., Nagy, Z., Luning Prak, E., and Weigert, M. (1995a). Immunoglobulin heavy chain gene replacement: a mechanism of receptor editing. Immunity *3*, 747–755.

Chen, C., Nagy, Z., Radic, M.Z., Hardy, R.R., Huszar, D., Camper, S.A., and Weigert, M. (1995b). The site and stage of anti-DNA B-cell deletion. Nature *373*, 252–255.

Cohn, M., and Langman, R.E. (1990). The protecton. Immunol. Rev. *115*, 7–142.

Coleclough, C. (1992). What determines the κ/λ ratio. Res. Immunol. *143*, 832.

Coleclough, C., Perry, R.P., Karjalainen, K., and Weigert, M. (1981). Aberrant rearrangements contribute significantly to the allelic exclusion of immunoglobulin gene expression. Nature *290*, 372–378.

Erikson, J., Radic, M.Z., Camper, S.A., Hardy, R.R., and Weigert, M.G. (1991). Expression of anti-DNA immunoglobulin transgenes in non-autoimmune mice. Nature *349*, 331–334.

Gay, D., Saunders, T., Camper, S., and Weigert, M. (1993). Receptor editing: an approach by autoreactive B cells to escape tolerance. J. Exp. Med. *177*, 999–1008.

Grawunder, U., Leu, T.M.J., Schatz, D.G., Werner, A., Rolink, A.G., Melchers, F., and Winkler, T.H. (1995). Down-regulation of *RAG1* and *RAG2* gene expression in preB cells after functional immunoglobulin heavy chain rearrangement. Immunity *3*, 601–608.

Gu, H., Forster, I., and Rajewsky, K. (1990). Sequence homologies, N sequence insertion and JH gene utilization in VHDJH joining: implications for the joining mechanism and the ontogenetive timing of Ly1 B cell and B-CLL progenitor generation. EMBO J. *9*, 2133–2410.

Ibrahim, S.M., Weigert, M., Basu, C., Erikson, J., and Radic, M.Z. (1995). Light chain contribution to specificity in anti-DNA antibodies. J. Immunol. *155*, 3223–3233.

Kofler, R., Duchosal, M.A., and Dixon, F.J. (1989). Complexity, polymorphism and connectivity of mouse Vκ gene families. Immunogenetics *29*, 65–74.

Luning Prak, E., and Weigert, M. (1995). Light chain replacement: a new model for antibody gene rearrangement. J. Exp. Med. *182*, 541–548.

Luning Prak, E., Trounstine, M., Huszar, D., and Weigert, M. (1994). Light chain editing in κ-deficient animals: a potential mechanism of B cell tolerance. J. Exp. Med. *180*, 1805–1815.

Nemazee, D. (1995). Does immunological tolerance explain the waste in the B-lymphocyte immune system? Experiment and Theory. Ann. NY Acad. Sci. *764*, 397–401.

Radic, M.Z., and Weigert, M. (1994). Genetic and structural evidence for antigen selection of anti-DNA antibodies. Annu. Rev. Immunol. *12*, 487–520.

Radic, M.Z., Mascelli, M.A., Erikson, J., Shan, H., and Weigert, M. (1991). Ig H and L chain contributions to autoimmune specificities. J. Immunol. *146*, 176–182.

Radic, M.Z., Erikson, J., Litwin, S., and Weigert, M. (1993a). B lymphocytes may escape tolerance by revising their antigen receptors. J. Exp. Med. *177*, 1165–1173.

Radic, M.Z., Mackle, J., Erikson, J., Mol, C., Anderson, W.F., and Weigert, M. (1993b). Residues that mediate DNA binding of autoimmune antibodies. J. Immunol. *150*, 4966–4977.

Shlomchik, M.J., Mascelli, M.A., Shan, H., Radic, M.Z., Pisetsky, D., Marshak-Rothstein, A., and Weigert, M. (1990). Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation. J. Exp. Med. *171*, 265–297.

Tiegs, S.L., Russell, D.M., and Nemazee, D. (1993). Receptor editing in self-reactive bone marrow B cells. J. Exp. Med. *177*, 1009–1020.